Extensively heated milk and egg as oral immunotherapy

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Purpose of review
The introduction of extensively heated milk and egg protein into the diet has been explored in recent years.

Recent findings
Studies have suggested that a large subset of children who react to unheated milk or egg can tolerate extensively heated forms of these foods. Immunologic changes induced by a diet containing baked milk and egg are similar to changes that have been observed during oral immunotherapy trials. The baked milk and egg diet appears to accelerate the development of regular milk and egg tolerance when compared with strict avoidance.

Summary
An oral challenge to extensively heated milk and egg into milk and egg allergic children's diets should be considered when appropriate. Oral food challenges are the most reliable means of establishing a diagnosis and should be undertaken under physician supervision.

Keywords
baked milk and egg, food allergy, heated milk and egg, oral immunotherapy

INTRODUCTION

Oral immunotherapy shows great promise in food allergy research; however, it is clear that food oral immunotherapy with native food protein may not be possible for some patients as there is a significant rate of adverse reactions, including severe respiratory reactions and gastrointestinal discomfort [1]. Therefore, alternative forms of food immunotherapy are being investigated [2]. One such alternative approach involving the introduction of extensively heated food protein has been explored in recent years. From these investigations, it appears that a large subset of children who react to unheated milk or egg tolerate extensively heated forms [3–6], and in fact, tolerance to extensively heated milk and egg may identify patients with more favorable prognosis. For this subset of children, the benefit of food oral immunotherapy may be low. Immunologic changes induced by a diet containing baked milk and egg are similar to the changes that have been observed during oral immunotherapy trials. Whether the continued ingestion of extensively heated milk and egg enables the immune system to develop tolerance to the unheated forms of these foods, and whether this tolerance is accelerated by the incorporation of these foods, remains to be seen. Recent studies have suggested that this is a possibility and mechanistic studies have provided insight on the prediction of tolerance (Table 1) [4–6,7▪,8–19].

EFFECT OF HEATING ON ALLERGENICITY: CONFORMATIONAL VERSUS SEQUENTIAL EPITOPES

Food processing can either decrease or increase protein allergenicity. Decrease in allergenicity is likely the result of the destruction of conformational epitopes, as well as chemical reactions between proteins, fats, and sugars in the food matrix that limit the accessibility of protein to the immune system. Conversely, an increase in allergenicity

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KEY POINTS

- A large (~70%) subset of children who react to milk or egg can tolerate extensively heated forms of these foods.
- Immunologic changes induced by a diet containing baked milk and egg are similar to changes that have been observed during oral immunotherapy trials.
- The baked milk and egg diet appears to accelerate the development of regular milk and egg tolerance when compared with strict avoidance.
- Oral challenges to extensively heated milk or egg should be considered for milk and egg allergic children. Oral food challenges are the most reliable means of establishing a diagnosis. It is most prudent to undertake these challenges under physician supervision with all standard precautions, because reactions to heated milk and egg may be severe.

may be the result of the formation of neoepitopes, increasing accessibility of existing epitopes by the effect of food matrix leading to decreased protein digestibility, or glycation [20].

High heat such as baking has been found to reduce the allergenicity of many food proteins, presumably by changing the conformation of heat-labile proteins that results in loss of conformational epitopes [20,21]. Given that children with transient egg and milk allergy produce IgE antibodies directed primarily against conformational epitopes that are destroyed during extensive heating or processing [16,17], it stands to reason that children who outgrow their allergy may have IgE that binds to conformational epitopes.

Cow's milk

Studies have demonstrated this concept in cow's milk allergic children. In a study involving 8 patients with persistent milk allergy and 11 patients who subsequently outgrew milk allergy, 5 sequential IgE-binding epitopes were not recognized by any of the patients with transient milk allergy, but were recognized by the majority of patients with persistent allergy in a SPOTS membrane assay [8]. In a similar study [11] of 33 children with milk allergy and 10 children who subsequently outgrew their milk allergy, children with persistent milk allergy had increased epitope diversity and higher affinity of IgE binding compared with those who outgrew milk allergy. In addition, binding to higher numbers of IgE peptides in a peptide microarray assay was associated with more severe allergic reactions during oral milk challenge.

Hen's egg

Similar findings have been reported in hen's egg allergic children. An early case study showed that sera from children with transient egg allergy had no IgE antibodies against reduced and alkylated (sequential epitopes) ovomucoid, whereas serum from children with persistent egg allergy recognized sequential ovomucoid epitopes, suggesting that both conformational and sequential B cell epitopes play a significant role in ovomucoid allergenicity [2,16]. In a study comparing 11 children with transient egg allergy to 7 children with persistent egg allergy, Jarvinen *et al.* showed that none of the patients with transient egg allergy had IgE antibodies directed against certain sequential epitopes of ovomucoid: amino acids 1–10, 11–20, 47–56, and 113–122. However, all seven patients with persistent egg allergy recognized at least one of these epitopes [2,17]. In a more recent study [19], ovomucoid-specific IgE detected by microarray appeared to be a good predictor of clinical egg allergy, as ovomucoid positive children had a high frequency of egg allergy, and ovomucoid negative children had a high frequency of tolerance to boiled egg.

DIFFERENTIAL EFFECT OF HEATING ON FOOD PROTEINS

Thermal processing can destroy IgE-binding conformational epitopes but usually does not destroy sequential IgE-binding epitopes, and this appears to play a role in milk and egg allergy. Food behavior under different heating conditions, as well as interactions with food matrix (e.g. as seen in the case of hen's egg white), are highly variable among different foods, and therefore, heating may not have similar effects for all foods. In addition, thermal processing may not simply alter IgE epitopes, as it can alter different biophysical and immunological properties of a food protein such as its structure, function, solubility, digestibility, and T-cell responses [22]. Some food proteins are rendered more allergenic by thermal processing. For peanut proteins, high temperature may enhance allergenicity as a result of glycation (Maillard reaction) that induces the formation of Ara h 2 aggregates that are more resistant to gastric digestion and bind IgE antibody more effectively than unheated Ara h 2 [22,23]. Beyer *et al.* [24] analyzed the peanut proteins using SDS-PAGE and immunoblotting with sera from eight peanut allergic patients, and showed that the relative amount of Ara h 1 was reduced in the fried and boiled preparations compared with roasted peanuts. There was also significantly less IgE binding to Ara h 2 and Ara h 3 in fried and

Table 1. Summary of studies on heated milk and egg

Study	Patient population and methods	Results
Milk studies – clinical		
Nowak-Węgrzyn *et al.* [5]	Children with milk allergy (positive SPT or detectable serum milk-specific IgE, and a history of an allergic reaction to milk; or milk-specific IgE levels or SPT highly predictive for clinical reactivity: if ≤2 years old, a level >5 kU_A/l; if >2 years old, a level >15 kU_A/l; SPT wheal diameter ≥8 mm)	One hundred children underwent heated milk challenges: 68 tolerated extensively heated milk only, 23 reacted to heated milk, and 9 tolerated both heated and unheated milk. Heated milk-reactive children had significantly larger SPT wheals and higher milk-specific and casein-specific IgE levels than other groups. At 3 months, children ingested heated milk had significantly smaller SPT wheals and higher casein-IgG_4 compared with baseline
Kim *et al.* [7▪]	Children evaluated for tolerance to baked milk (muffin) underwent sequential food challenges to baked cheese (pizza) followed by unheated milk; immunologic parameters were measured at challenge visits. Comparison group was matched to active children to evaluate the natural history	Among 65 children initially tolerant to baked milk, 39 (60%) now tolerate unheated milk, 18 (28%) tolerate baked milk/baked cheese, and 8 (12%) chose to avoid milk strictly. Among the baked milk-reactive group ($n=23$), 2 (9%) tolerate unheated milk, 3 (13%) tolerate baked milk and baked cheese, whereas the majority (78%) avoid milk strictly. Children who were initially tolerant to baked milk were 28 times more likely to become unheated milk tolerant compared with baked milk-reactive children ($P<0.001$); children who incorporated dietary baked milk were 16 times more likely than the comparison group to become unheated milk tolerant ($P<0.001$)
Milk studies – mechanistic		
Jarvinen *et al.* [8]	Eleven patients with persistent milk allergy and eight patients who subsequently outgrew their milk allergy. Twenty-five decapeptides of α_{s1}-casein, α_{s2}-casein, κ-casein, α-lactalbumin, and β-lactoglobulin, comprising the core epitopes, synthesized on a SPOTs membrane. Sera from individual patients were used for immunolabeling	Five IgE-binding epitopes were not recognized by any of the patients with transient milk allergy but showed binding by the majority of the patients with persistent allergy. Antibodies against at least one of the epitopes were identified in all patients with persistent milk allergy
Shreffler *et al.* [9]	Allergic ($n=8$), heated milk-tolerant ($n=18$), outgrown ($n=7$), or control ($n=7$) pediatric children were defined by oral food challenge. PBMCs were cultured with caseins and controls, and proliferating $CD25^+CD27^+$ Treg cells were identified by flow cytometry. In addition, cells were characterized for their expression of FoxP3, CTLA 4, CD45R0, and CD127	A higher percentage [median (25th% to 75th%), 16.85% (7.1–31.7)] of proliferating allergen-specific $CD25^+CD27^+$ T cells from cultures of heated milk-tolerant children [4.91% (2.6–7.5)] than those with allergy. Controls with no history of milk allergy had low percentages of these cells [2.9% (2.4–6.0)]. Outgrown children had intermediate percentages [9.0% (2.7–16.4)]
Wanich *et al.* [10]	Allergic, heated milk-tolerant, outgrown, or control pediatric individuals were defined by oral food challenge. Whole-blood cells were stimulated *in vitro* with milk allergen doses in the presence or absence of autologous serum. Activated basophils were identified by means of flow cytometry	Heated milk-tolerant individuals' basophils were significantly less responsive to milk allergen stimulation at all doses than were basophils from heated milk-reactive individuals
Wang *et al.* [11]	Thirty-three children with milk allergy and eight children who outgrew milk allergy. Peptides, consisting of 20 amino acids overlapping by 17 and corresponding to the primary sequences of α_{s1}-caseins, α_{s2}-caseins, β-caseins, and κ-caseins and β-lactoglobulin were arrayed on glass slides	Children with milk allergy had increased epitope diversity compared with those who outgrew milk allergy. Binding to higher numbers of IgE peptides was associated with more severe allergic reactions during the challenge. In a competitive microarray assay, allergic patients demonstrated a combination of high and low-affinity IgE binding, whereas children who had outgrown their milk allergy had primarily low-affinity binding
Caubet *et al.* (in revision)	Two cohorts of milk allergic children were prospectively recruited: 97 children from a study on tolerance to baked milk (recruited 2004–2207), and a second cohort of 128 children (recruited 2008–2010)	The two cohorts of milk allergic children demonstrated the levels of IgE to cow's milk, casein and β-lactoglobulin were significantly higher in baked milk-reactive patients compared with baked milk-tolerant patients. Casein-specific IgE had the highest positive and negative predictive values compared with specific IgE to cow's milk or β-lactoglobulin, and casein-specific and β-lactoglobulin specific IgE/IgG_4 ratios were significantly higher in baked milk-reactive children with compared with baked milk-tolerant children

Table 1 (*Continued*)

Study	Patient population and methods	Results
Egg studies – clinical		
Urisu *et al.* [6]	Double-blind, placebo-controlled, food challenges were performed in children with high IgE antibodies for egg white. Challenge foods included freeze-dried, heated (liquid egg white heated at 90°C for 60 min), and heated and ovomucoid-depleted egg white (liquid egg white heated at 90°C for 60 min, then squeezed through bag made of cotton cloth to remove ovomucoid)	Twenty one of 38 children with positive challenge responses to freeze-dried egg white had negative challenge responses to heated egg white. Sixteen of 17 children with positive responses to heated egg white did not respond to heated and ovomucoid-depleted egg white challenge. IgE antibody levels to ovomucoid were significantly higher in children with positive responses to a challenge with heated egg white than those with no response
Des Roches *et al.* [12]	Sixty children allergic to eggs (history of clinical reaction to egg with positive SPT) performed oral food challenge to cooked eggs (cake)	Seventy-three percentage of the egg-allergic children tolerated egg in its cooked form. SPT $\geq$10mm appears to be a marker associated with reaction to cooked egg challenge ($P=0.03$). Presence of multiple food allergies seems to increase the risk of reacting to cooked egg ($P=0.04$)
Konstantinou *et al.* [13]	Ninety-four children (55 with diagnosed of hen's egg allergy by positive challenge result, convincing history combined with positive specific IgE level, or both; 39 sensitized to hen's egg by positive SPT to egg white, egg white specific IgE, or both) were retrospectively evaluated. Children were challenged with increasing quantities of a cake baked with one egg; negative challenge results were followed by instructions to continue the consumption with a slow and gradual increase of egg content to 1.5 g of egg protein. An open challenge to egg was subsequently performed	More than 90% of children could tolerate baked egg in a cake (92.7% of children with egg allergy and 92.3% of sensitized children). After 6 months, 87 children who were freely receiving baked egg underwent open food challenges to whole egg; only 4 (4.6%) of them had positive challenges to whole egg
Ando *et al.* [14]	Children with suspected egg allergy underwent double-blind, placebo-controlled food challenges with raw and heated egg (heated liquid egg white at 90°C for 60 min, freeze dried, then homogenized into powder)	Of 108 patients, 38 patients reacted to heated egg (considered allergic to raw and heated egg), 29 patients reacted only to raw egg white, and 41 patients were tolerant. For reaction to heated egg white, the positive decision point for ovomucoid ImmunoCAP was 10.8 kU_A/l and the negative decision point was 1.2 kU_A/l
Lemon-Mule *et al.* [4]	Children with egg allergy (history and positive SPT to egg white and detectable serum egg white-specific IgE, or a positive oral food challenge to egg, or egg-specific IgE level >7 kU_A/l in children older than 2 years of age or greater than 2 kU_A/l in children less than 2 years of age) underwent physician supervised oral food challenges with extensively heated egg (muffin and waffle), with tolerant children undergoing regular egg challenges	64 of 117 children tolerated heated egg, 23 tolerated regular egg, and 27 reacted to heated egg. Heated egg-reactive children had larger skin test wheals and greater egg white-specific, ovalbumin-specific, and ovomucoid-specific IgE levels compared with heated egg- and egg-tolerant children. Continued ingestion of heated egg associated with decreased skin test wheal diameters and ovalbumin-specific IgE levels and increased ovalbumin-specific and ovomucoid-specific IgG_4 levels
Nowak-Wegrzyn *et al.* [29]	Children with documented IgE-mediated egg allergy who tolerated baked egg challenge (in form of muffin and waffle) incorporated baked egg into their diet. Children who were initially baked egg reactive were re-challenged every year	Seventy-nine children in the active arm were followed for a median of 37.8 months. Overall, 70 (89%) tolerated baked egg, and 42 (53%) now tolerate regular egg. Of 23 initially baked egg-reactive children, 14 (61%) eventually tolerated baked egg and 6 (26%) went on to tolerate regular egg. Children who were initially baked egg tolerant were 3.3 times more likely to develop regular egg tolerance than children who were initially baked egg reactive
Clark *et al.* [15]	A longitudinal study from 2004 to 2010 of egg-allergic children who underwent challenge with well cooked, and if negative, uncooked egg	The median age at which tolerance occurred was 67 months for well cooked and 127 months for uncooked egg. The hazard ratio was 2.23, indicating that children outgrew allergy to well cooked egg approximately twice as quickly as they outgrow allergy to uncooked egg
Caubet *et al.* [27]	One hundred and seven egg-allergic children were challenged to baked egg, and the outcomes of these challenges were related to the level of specific IgE and IgG_4 to ovalbumin and ovomucoid, component IgE/IgG_4 ratios, and mediator release in a functional assay based on rat basophil leukemia line	Baked egg-reactive children had significantly higher ovalbumin and ovomucoid ratios of IgE/IgG_4 and mediator release in the rat basophil leukemia-based assay than did tolerant children

Table 1 (*Continued*)

Study	Patient population and methods	Results
Egg studies – mechanistic		
Cooke and Sampson [16]	Children with persistent egg allergy and atopic dermatitis: ovomucoid dodecapeptides overlapping by 10 amino acids were synthesized on a SPOTs membrane	Serum from a children with transient egg allergy had no IgE antibodies against reduced and alkylated (sequential epitopes) ovomucoid, whereas serum from a children with persistent egg allergy recognized sequential ovomucoid epitopes
Jarvinen *et al.* [17]	Eleven children with transient and seven children with persistent egg allergy: central decapeptides from each of the major sequential IgE-binding epitopes of ovomucoid synthesized on a SPOTs membrane: immunolabeling was done with individual patients' sera	Both groups had comparable ranges of egg-specific IgE levels, but none of the patients with transient egg allergy had IgE antibodies against these epitopes of ovomucoid: amino acids 1–10, 11–20, 47–56, and 113–122. All seven patients with persistent egg allergy recognized at least four of these immunodominant epitopes
Martos *et al.* [18]	Mice were orally sensitized with ovalbumin or ovomucoid and challenged with native or heated proteins to evaluate their allergenicity. Immunoreactivity was assessed by immunoblotting egg-allergic children's sera; in-vitro gastrointestinal digestion of native and heated ovalbumin and ovomucoid was studied with SDS-PAGE and liquid chromatography. Human intestinal epithelial cells were investigated for intestinal uptake of intact native and heated ovalbumin and ovomucoid. Rat basophil leukemia cells (passively sensitized with mouse serum) and human basophils (passively sensitized with serum from children with egg allergy) were used to assess effector cell activation by heated, digested, and transported ovalbumin and ovomucoid	Heated ovalbumin and ovomucoid did not induce symptoms of anaphylaxis in sensitized mice when administered orally. Heating did not completely destroy IgE-binding capacity of ovalbumin or ovomucoid but enhanced in-vitro digestibility of ovalbumin. Mediator release in rat basophil leukemia assay and basophil activation were diminished by digestion of both ovalbumin and ovomucoid. Transport across human intestinal epithelial cells in a form capable of triggering basophil activation or T-cell activation was prevent by heating of allergens
Alessandri *et al.* [19]	Sixty-eight children referred for suspected egg allergy underwent double-blind, placebo-controlled food challenge with boiled (boiled for 10 min) and raw eggs. IgE to egg allergens available on the immunosolid phase allergen chip (ISAC) 103 microarray were run	Forty-four of forty-seven (94%) of Gal d 1 negative patients tolerated boiled egg, whereas 20 of 21 (95%) of Gal d 1 positive patients reacted to raw egg

boiled peanuts compared with that in roasted peanuts, although the amount of protein was similar in the preparations [24]. Roasting may also increase the efficiency of Ara h 1 extraction and cause antibody-binding epitopes to be more accessible, as one study demonstrated that Ara h 1 levels were up to 22-fold higher in roasted as compared with raw peanuts [25]. Similar findings have been found with other foods. With crustaceans, one study [26] showed that more patients were identified using boiled extracts of shrimp and American and spiny lobsters than with raw extracts; in addition, wheal sizes of the skin test reactions and specific IgE levels were also significantly greater using boiled extracts.

CLINICAL TRIALS WITH EXTENSIVELY HEATED MILK AND EGG

Several clinical trials have investigated the tolerance of heated milk and egg in children with milk and egg allergy [4–6,14]. In a study by Nowak-Węgrzyn *et al.* [5], children aged 2–17 years with milk allergy were challenged with extensively heated milk products (muffin and waffle baked with milk). Sixty-eight of ninety-one (75%) of children with milk allergy were able to tolerate baked milk product but not unheated milk. The 25% who reacted to baked milk had significantly larger skin-prick test wheals and higher milk-specific and casein-specific IgE levels than other groups. At 3 months, children ingesting baked milk products had significantly smaller skin-prick test wheals and higher casein-sIgG$_4$ compared with baseline.

In a study by Urisu *et al.* [6], 72 patients with high IgE antibody titers to egg white completed a double-blinded, placebo-controlled, food challenges to freeze-dried, heated, and heated/ovomucoid-depleted egg white. Twenty-one of thirty-eight patients with positive challenge responses to freeze-dried egg white had negative challenge responses to heated egg white, whereas 16 of 17 patients with positive responses to heated egg white did not react to heated and ovomucoid-depleted egg white. This study demonstrates that a significant number of children reactive to freeze-dried egg could tolerate heated egg, and suggests that the heated and ovomucoid-depleted egg white preparation was less allergic than the heated or freeze-dried preparations.

In a clinical trial by Lemon-Mule *et al.* [4] on extensively heated (baked egg) egg diet, 64 of 117 (55%) patients with median age of 6.9 years (range, 1.6–18.6 years) tolerated baked egg, 23 tolerated regular egg (lightly heated, e.g. scrambled egg or French toast), and 27 reacted to baked egg. Baked egg-reactive patients had larger skin test wheals and higher egg white-specific, ovalbumin-specific, and ovomucoid-specific IgE levels compared with baked egg and regular egg-tolerant patients. As seen in the baked milk study, continued ingestion of baked egg was associated with decreased skin test wheal diameters, ovalbumin-specific IgE levels, and increased ovalbumin-specific and ovomucoid-specific IgG_4 levels. In a study by Ando *et al.* [14] of 108 patients with a median age of 34.5 months (range, 14–156 months), 38 patients reacted to heated egg (heated to 90°C for 60 min; these patients were considered allergic to raw and heated egg), 29 patients reacted only to raw egg white (tolerated heated egg), and 41 patients were tolerant to raw egg white.

A majority of children with milk and egg allergy tolerated baked milk and egg products during the initial physician-supervised oral challenge. In the milk study by Nowak-Węgrzyn *et al.* [5], reactions that were treated with epinephrine occurred only in children who reacted to baked milk products, but not in children who tolerated baked milk and reacted to unheated milk. This suggests that tolerance to baked milk is a marker of a milder milk allergy that is likely to be outgrown. In contrast, in the egg study [4], there were equal proportions of children who received epinephrine during baked and regular egg challenges, indicating that tolerance to baked egg is not predictive of milder clinical reactions to regular egg.

Skin-prick tests and serum IgE levels were not reliable for identifying children tolerant to baked milk or egg in these studies; oral food challenges were required to determine the tolerance. Serum IgE levels, however, offered some clinically relevant information, as the majority of children who reacted to extensively heated milk had milk-specific IgE antibody levels greater than 35 kU_A/l [5]. In a study by Caubet *et al.* (in preparation), two cohorts of milk-allergic children demonstrated the levels of IgE to cow's milk, casein, and β-lactoglobulin were significantly higher in baked milk-reactive patients compared with baked milk-tolerant patients. In addition, casein-specific IgE had the highest positive and negative predictive values compared with specific IgE to cow's milk or β-lactoglobulin, and casein and β-lactoglobulin specific IgE/IgG_4 ratios were significantly higher in baked milk-reactive patients when compared with baked milk-tolerant patients (Caubet *et al.*, in revision). In the study by Ando *et al.* conducted in a cohort of Japanese children, the positive decision point for reactivity to heated egg was 10.8 kU_A/l for ovomucoid ImmunoCAP and the negative decision point was 1.2 kU_A/l (UniCAP, Phadia, Uppsala, Sweden). In another study by Caubet *et al.* [27], baked egg-reactive children were found to have significantly higher ovalbumin and ovomucoid ratios of IgE/IgG_4 and higher mediator release in the functional assay based on the rat basophil leukemia cells than tolerant children. Although further investigation is needed, using IgE and IgG_4 to ovalbumin and ovomucoid to predict reactivity to baked egg is a future possibility [27].

BAKED MILK AND EGG AS ORAL IMMUNOTHERAPY

Some studies have examined the development of tolerance to milk and egg in patients eating heated milk and egg products.

Cow's milk

In a prospective study [7▪] reporting on the outcomes of children who incorporated baked milk products into their diets, the addition of baked milk to the diet of children who tolerate such foods appears to accelerate the development of unheated milk tolerance compared with those who avoided milk strictly. In this study, 88 children underwent challenges at varying intervals (range, 6–54 months) over a median of 37 months (Fig. 1). Among 65 patients who initially tolerated baked milk, 39 (60%) went on to tolerate unheated milk, 18 (28%) tolerate baked milk and baked cheese, and 8 (12%) avoid milk strictly. Among the baked milk-reactive subgroup of 23 patients, 2 (9%) went on to tolerate unheated milk, 3 (13%) tolerated baked milk and baked cheese, and 78% avoided milk strictly. Patients who were initially tolerant to baked milk were found to be 28 times more likely to become unheated milk tolerant, compared with baked milk-reactive patients ($P < 0.001$). Patients who incorporated dietary baked milk were 16 times more likely than a retrospectively assessed comparison group (that followed standard advice for strict avoidance of dietary milk) to become tolerant to unheated milk ($P < 0.001$) [7▪]. Also of note was that the median casein IgG_4 levels in the baked milk-tolerant group increased significantly, although the median milk IgE levels did not change significantly [7▪]. These data support the hypothesis that tolerance to baked milk is a marker of transient IgE-mediated cow's milk allergy, whereas reactivity to baked milk portends a more persistent phenotype.

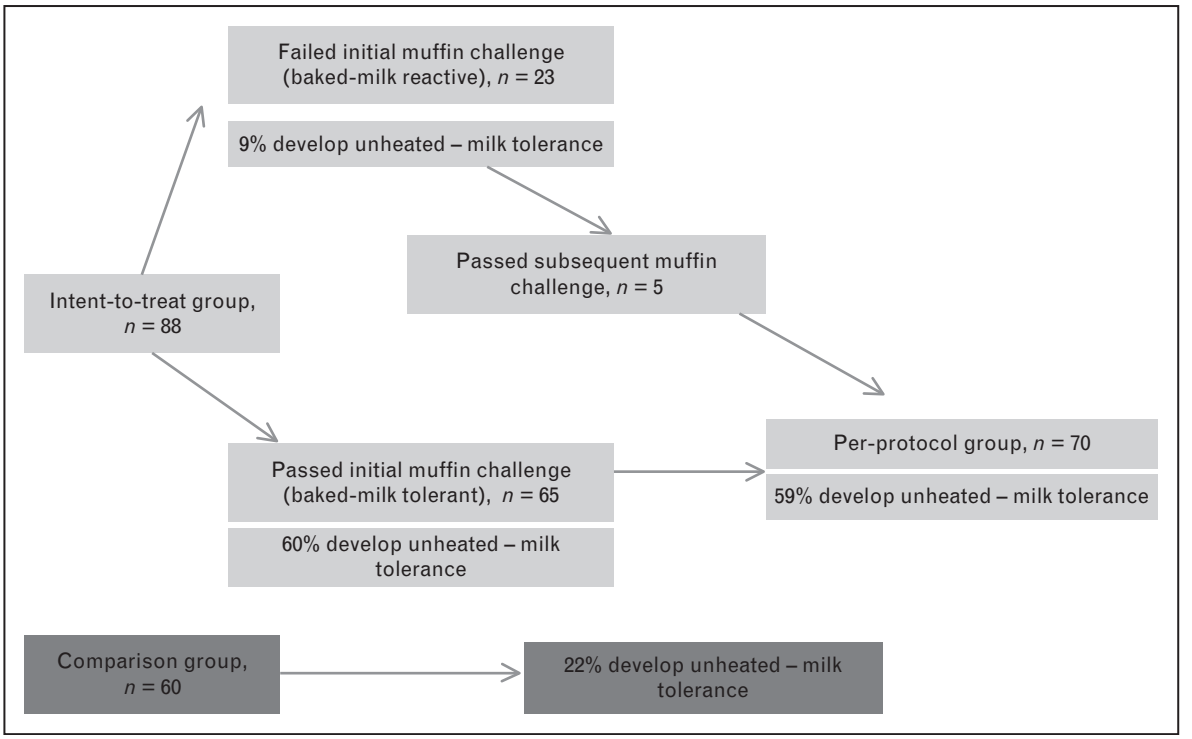

FIGURE 1. Study design and enrollment for baked milk clinical trial. Reproduced with permission [7▪].

Hen's egg

In a retrospective study [28], a higher proportion of those who outgrew egg allergy, when compared with those with persistent egg allergy, reported ingestion of baked egg as part of their diet; however, this difference did not achieve statistical significance. In a longitudinal study by Clark *et al.* [15], children with egg allergy underwent challenge with well cooked, and, if negative, uncooked egg. They found that tolerance was gained twice as rapidly to well cooked than uncooked egg. In a prospective study that is in press, egg-allergic children who tolerated baked egg challenge (in the form of muffin and waffle) incorporated baked egg into their diet, and patients who were initially baked egg reactive were re-challenged every 12 months (Fig. 2) [29]. Seventy-nine patients in the active arm were followed for a median of 37.8 months; overall, 70 children tolerated baked egg and 42 now tolerate regular egg. Of the 23 initially baked egg-reactive patients, 14 (61%) eventually tolerated baked egg and 6 (26%) went on to tolerate regular egg. Patients who were initially baked egg tolerant were 3.3 times more likely to develop regular egg tolerance than those who were initially baked egg reactive. Patients in the active per-protocol group were 14.6 times (95% confidence interval, 5.8–36.4; $P < 0.0001$) more likely to develop regular egg tolerance than those in a retrospectively assessed comparison group who continued strict avoidance of egg and more likely to develop the tolerance earlier (median 22.0 versus 66.7 months; $P < 0.0001$). These findings suggest that the baked egg diet accelerates the development of regular egg tolerance when compared with strict avoidance [29].

OTHER POSSIBLE MECHANISMS

Aside from the serologic changes noted (increased in IgG_4 antibody levels and decreases in SPT wheal) in patients who tolerate extensively heated milk or egg, studies on basophil reactivity, regulatory T cells, and the Maillard reaction have been completed, suggesting involvement of these mechanisms in food allergy.

Increased basophil reactivity

A study by Wanich *et al.* [10] found that children who reacted to baked milk had significantly higher basophil reactivity to stimulation with casein when compared with that seen in children who tolerated baked milk.

Regulatory T cells

In a study by Shreffler *et al.* [9], children tolerant of baked milk had more regulatory T cells in the peripheral blood at baseline. Following the introduction of baked milk, the fraction of peripheral T cells

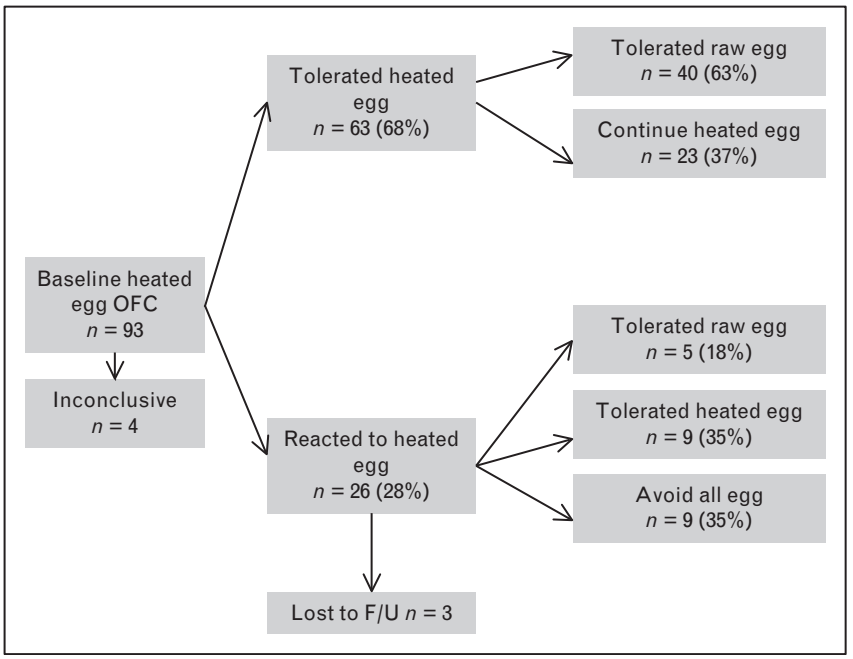

FIGURE 2. Study design and enrollment for baked egg trial. Adapted with permission from [29].

decreased, which suggested that these cells might have migrated to the site of contact with dietary antigen.

Enhanced gastrointestinal digestibility

Heat treatment is thought to reduce the allergenicity of ovalbumin and ovomucoid by various mechanisms. In a mouse model study by Martos *et al.* [18], heated ovalbumin was shown to have enhanced gastrointestinal digestibility. The absorbed forms of heated ovomucoid were not capable of triggering basophils, suggesting additional degradation of heated ovomucoid during the intestinal uptake.

The Maillard reaction occurs between reducing sugars and proteins during thermal food processing, and produces chemically glycated proteins termed advanced glycation end-products (AGEs). Ilchmann *et al.* [30] demonstrated that the formation of AGEs enhances the $CD4^+$ T-cell immunogenicity of a food allergen, suggesting that the influence of the Maillard reaction needs to be considered when evaluating the immunogenicity of food allergens.

COMPARISON BETWEEN ORAL IMMUNOTHERAPY AND BAKED MILK AND EGG DIET

Although food oral immunotherapy is very promising, some children with food allergy may not be able to undergo this type of treatment because of the risk of adverse events. In food oral immunotherapy trials, side-effects include but are not limited to oral pruritus, respiratory symptoms, gastrointestinal symptoms such as abdominal discomfort, emesis and diarrhea, as well as reactions requiring the use of epinephrine [31–42]. The potential for the development of eosinophilic gastrointestinal disease such as eosinophilic esophagitis (EoE) is also a concern [35]. In addition, augmentation factors such as exercise, menstruation, and viral illnesses often result in the need for dose adjustment, which may further limit the patient population for which food oral immunotherapy is a feasible choice [41]. A diet containing baked milk and egg may be a safer and perhaps more natural approach to oral immunomodulation for these foods. Growth parameters, intestinal permeability, as well as chronic conditions such as asthma, atopic dermatitis, and allergic rhinitis were not worsened during baked milk or egg diet [4,5,7▪]. In addition to the immunomodulatory benefits, the inclusion of extensively heated milk and egg to the child's diet may greatly improve the quality of life, vastly increasing the variety of food products that may be consumed. This would facilitate the fulfillment of nutritional requirements, reduce parental anxiety, lessen the child's discomfort in social situations, and possibly provide an effective strategy to shorten the time to achieve tolerance. Until further studies have been completed to establish the overall safety and efficacy of this method, the introduction of baked milk and egg should be approached with caution and should be done under physician supervision. However,

after a baked milk or egg challenge is passed under supervision, the extensively heated form of milk or egg may be continued at home, eaten as a part of the child's regular diet.

LIMITATIONS

Further clinical as well as mechanistic studies are needed to characterize the additional factors associated with nonreactivity of extensively heated milk and egg. The clinical trials that have been carried out thus far do not include infants and young children, particularly those less than 2 years of age. Thus, the results of these studies should be applied with caution to this population. The ongoing rigorous food oral immunotherapy trials include an additional step to distinguish between the desensitization that depends on the regular intake of the food and permanent oral tolerance that does not depend on food intake [43,44]. This involves removing the food from the diet for at least 1–2 months and performing the so-called tolerance food challenge. About 20–30% of treated children fail this challenge and are considered to be desensitized but not tolerant. The baked milk and egg studies did not incorporate a step for testing the permanence of tolerance in children who progressed to unheated milk and regular egg while on a baked milk and egg diet. This issue is being addressed in an ongoing baked milk trial (unpublished data).

Although limited, there are data that patients not exposed to cow's milk protein residue achieve tolerance to cow's milk earlier than patients who follow an extensively hydrolyzed cow's milk diet [45].

At this point in time, the view that early and deliberate exposure to allergenic foods may prevent or delay the onset of food allergy is one that is not substantiated by the basic and clinical research that has been completed [46]. Likewise, further large, prospective, randomized controlled clinical trials must be carried out to provide the conclusive evidence comparing the effects of baked milk and egg diet versus strict dietary avoidance of milk and egg on development of tolerance to these foods.

CONCLUSION

In view of the diverse benefits but also possible risks of the baked milk or egg diet, introduction of extensively heated milk and egg should be considered. Current routine laboratory diagnostic tests do not reliably predict tolerance to extensively heated milk or egg; therefore, oral food challenges remain the ultimate means of establishing a diagnosis. These challenges must be undertaken under physician supervision with all precautions typically used for performing food challenges in children, because reactions to heated milk and egg may be severe.

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None.

Conflicts of interest

No conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

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